Iranian Journal of Veterinary Science and Technology
Vol. 7, No. 2, 2015, 63-74

IJVST

Growth promoting effects of a multi-strain probiotic on common carp (*Cyprinus carpio*) fingerlings

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Received:December 6, 2015 Accepted: May 15, 2016

Abstract

Probiotics have shown beneficial effects on growth parameters of various food animals. In this research, the growth promoting effects of a multi-strain probiotic was investigated on common carp (*Cyprinus carpio*). Accordingly, 200 fish (6-7g) were randomly allocated to 4 experimental groups. The first group received placebo and was served as the negative control. The second group received the prebiotic Fermacto at 3g/kg diet and was considered as the positive control. The remaining 2 groups received a multi-strain probiotic (PTX) at 75 and 150mg/kg feed, respectively. The body weights and lengths of all fish were measured at the end of the experiment (day 45). The amounts of protein, fat, ash and dry matter of fish meat were analyzed in all experimental groups (n= 6 each). The probiotic, at 75mg/kg feed, significantly increased the body weights and lengths, as well as the condition factor. Consistently, the feed conversion rate was lower in PTX treated groups. The percentage of meat fat was significantly lower in groups receiving either the probiotic at 150mg/kg feed, or Fermacto, compared to the control. The fish receiving PTX at 150mg/kg feed showed significantly higher amounts of meat protein. The levels of meat ash and meat dry matter were not statistically different compared to the control group. The present study suggests that PTX, at 75mg/kg feed, is effective in improving the growth and feed conversion rates of common carps.

Keywords: multi-strain probiotic, Fermacto, *Cyprinus carpio*, feed conversion rate

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Introduction

Antibiotics have been widely used in domestic animals as feed additives in order to promote growth and prevent infectious diseases. The use of antibiotics in animals, however, is associated with a variety of disadvantages such as drug residuals (Mehdizadeh *et al.*, 2010) and increased drug resistance (Devirgiliis, *et al.*, 2013). Therefore, a wide variety of alternative supplements including supplementary enzymes (Forster, *et al.*, 1999, Kazerani and Shahsavani, 2011), prebiotics (Lenoir-Wijnkoop, *et al.*, 2007) and probiotics (Reid and Friendship, 2002) have been introduced to the market.

A probiotic, by definition, is “a live microbial feed supplement e which beneficially affects the host animal by improving its microbial balance” (Fuller, 1989). Probiotics are believed to bring about a wide variety of beneficial effects for the host animal including enhanced immunity against pathogens, improved growth and body composition, and optimization of gut morphology and microbial balance (Gatesoupe, 1999, Ezendamand van Loveren, 2006, Merrifield *et al.*, 2010).

The fresh water fish, common carp (*Cyprinus carpio*), is widespread in eutrophic lakes and large rivers in Europe and Asia. It is also cultivated in growing ponds and is the most produced fish worldwide. Common carp is omnivorous. The fish can eat water plants, but it prefers to scavenge the bottom for insects, benthic worms, crustaceans (including zooplanktons) and crawfish. Common carps are usually stocked with other cyprinid species in poly-cultural systems. Manure or fertilizer is usually used to boost natural food production in semi-intensive ponds. In addition, supplementary feeding using cereals and/or pellets are also used in intensive production systems (Billard, 1999).

Despite vast research on probiotics in some domestic animals, little is known regarding the use of these supplements in common carps. Therefore, the main aim of this research was to investigate the growth promoting effects of a multi-strain probiotic (PTX) on common carps.

Materials and methods

The animals

Fingerling common carps were purchased from the market and were transferred to the Aquatic Animals’ Laboratory. The fish were sampled for probable microbial or parasitic infections after arrival. The animals were disinfected using formaldehyde 1/4000 solution for 15 min. All fish were diagnosed as healthy following clinical and paraclinical examinations prior to the commencement of the study. The fish were allowed to acclimate for 5 days before the start of the experiment.

Experimental tanks

The experiment was carried out in four glass aquaria. The volume of water in each tank was 150 liters. Water exchange was carried out every other day. Proper aeration was achieved through using air pumps. The oxygen level in water was 5.8-6 ppm. Water temperature was maintained at 22°C throughout the experiments. The tanks were randomly allocated to four different experimental groups.

The supplements

A commercial multi-strain probiotic, Protexin (Probiotic International Ltd, UK), was used in this study. The supplement’s microbial composition is shown in Table 1. The recommended dose is 150 mg/kg feed for broilers and is claimed to contain a total of 2.09×10^9micro-organisms per gram.

Fermacto (PetAg Inc., USA), a commercial supplement with prebiotic effect, was used as the positive control treatment. According to the supplier, it is derived from an active fermentation of a primary *Aspergillus sp.* and contains high levels of mycelial fiber that promotes the growth of intestinal microflora.

According to the producer, this totally dead product was composed of crude protein (min 12%), crude fat (min 1.1%), mycelium fiber (max 45%) with less than 2% ash. The recommended dose for both poultry and fish is 0.3% of finished feed.

Experimental groups

A total of 134 fish, with an average body weight of 7.44 g, were randomly allocated to 4 different experimental groups. The first group received placebo (explained later) and served as the control. The second group was considered as the positive control and received a commercially available growth promoting supplement, Fermacto at 3g/kg feed (as recommended by the supplier). The remaining two groups received PTX at 75 and 150 mg/kg feed, respectively.

The diets and feed treatment

The fish received feed (Table 2), daily, at 3% of their initial body weight for 18 days. This amount was increased to 5% of the initial weights during the18th day to the 45th day of the experiment. PTX was thoroughly mixed with the moistened diets at 75 or 150 mg/kg feed. Fermacto was also thoroughly mixed with the diet in a similar manner at 3g/kg feed. The moistened pellets were then covered with kitchen oil (0.5ml/10g feed), so that the supplements are not washed out in the aquaria. The same procedure was used for the placebo, but no supplement was added.

Table 1. Microbial composition of the probiotic (PTX) according to the producing company.

Microorganism	CFU/g
Lactobacillus plantarum	1.28×10^8
Lactobacillus delbrueckii	2.22×10^8
Lactobacillus acidophilus	2.14×10^8
Lactobacillusrhamnosus	2.28×10^8
Bifidobacteriumbifidum	2.10×10^8
Streptococcus silivarius	4.18×10^8
Enterococcus faecium	5.60×10^8
Aspergillusoryzae	5.60×10^7
Candida pintolopepsii	5.68×10^7
Total count	2.09×10^9

CFU: colony forming unit

Table 2. The formula of the commercial diet (Starter SFT4, Beta Group of Hormoz Dam, Hormozgan) used in this study.

Composition	Quantity	Composition	Quantity
Crude protein	45±2%	Calcium	3±2%
Digestible protein	40±2%	Phosphor	2±1%
Crude energy (J)	4500±200	Total minerals	16±3%
Digestible energy (J)	4000±200	Free nitrogen(mg/kg)	<75
Crude fiber	<3%	Peroxide(mg/kg)	<10
Fat	13±2%	Vitamin A(IU)	4000
Lysine	4%	Vitamin D3(IU)	3000
Arginine	3.5%	Humidity	<10%

The experimental procedure

The carps were fed diets containing PTX, Fermacto or the placebo thrice a day for 45 days. All the fish were weighed, and their lengths were also measured at the end of the experiment at the 45th day. A total of 6 fish out of each group were randomly selected for measurements of meat protein, fat, dry matter and ash. The condition factor was also calculated according to the following formula (Deguara *et al.*, 1999):

Condition factor = fish weight (g) / body length (cm) × 100

It is noteworthy that no mortality occurred during the experiment.

Statistics

Statistical analysis and drawing the graphs were carried out using GraphPad Prism V5.0 (GraphPad Software, USA). The data were analyzed using one-way analysis of variance followed by Dunnett's Multiple Comparison Test. Unless mentioned otherwise, all data are represented as means and SEM.

Results

At the end the experiment, the average body weights of all treated groups were higher than that of the control group. However, the best significant result was observed in fish receiving PTX at 75mg/kg feed, with 70% higher average body weight, compared to the control group (Figure 1). The average body lengths of different experimental groups showed a rather similar pattern, being significant only with the lower dose of the probiotic (Figure 2).

The condition factor was significantly higher in PTX treated fish, but the highest effect was achieved with 75mg/kg (Figure 3).

The feed conversion rate (FCR) was highest in the control group (Table 3). It declined by up to 62% and 38% in groups receiving the probiotic at 75 and 150mg/kg feed, respectively.

The highest average percentage of meat fat was observed in the control group (Figure 4). The fish received PTX at 150mg/kg feed had an average meat fat 54% lower than that of the control.

Table 3. The feed conversion rate (FCR) of the fish treated with the probiotic (PTX), Fermacto (as positive control) or placebo (control) following 45 days of the experiment.

Groups	Control	Fermacto	PTX	
			75 mg/kg	150 mg/kg
FCR	**2.33**	**1.90**	**0.88**	**1.45**

FCR: feed conversion rate

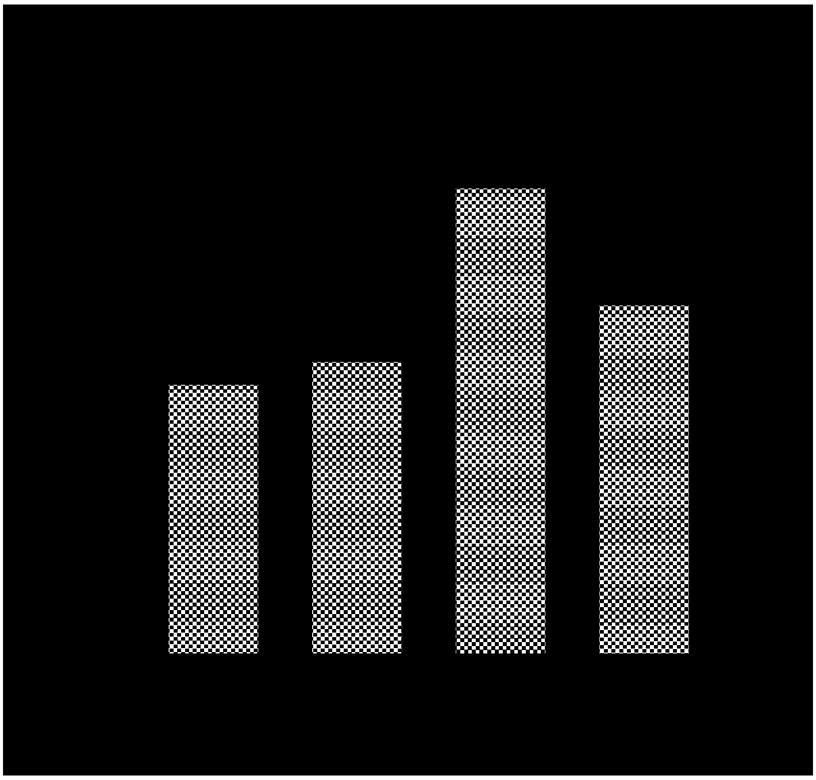


Figure 1.The effect of the multi-strain probiotic (PTX) on fish body weights following 45 days of the experiment. Data are represented as means and SEM. The asterisks indicate statistical significance compared to the control (*:*p*<0. 05, *: *p*<0. 001).**

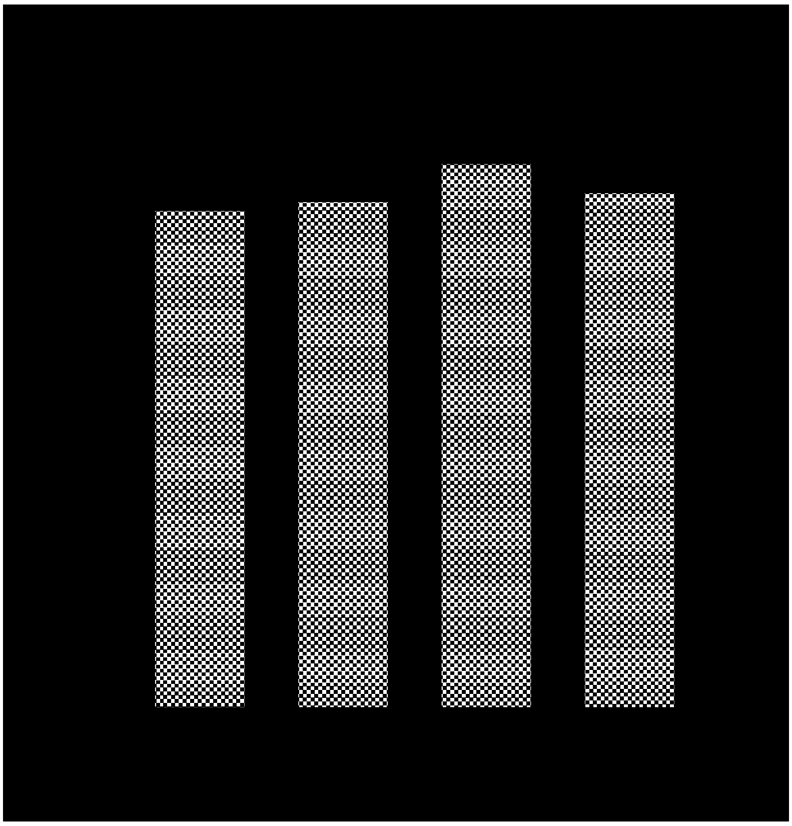


Figure 2.Body lengths of the fish treated by PTX, Fermacto (used as positive control) or placebo (control) following 45 days of the experiment. Data are represented as means and SEM. The asterisk indicates significant difference compared to the control (*p*<0. 01).

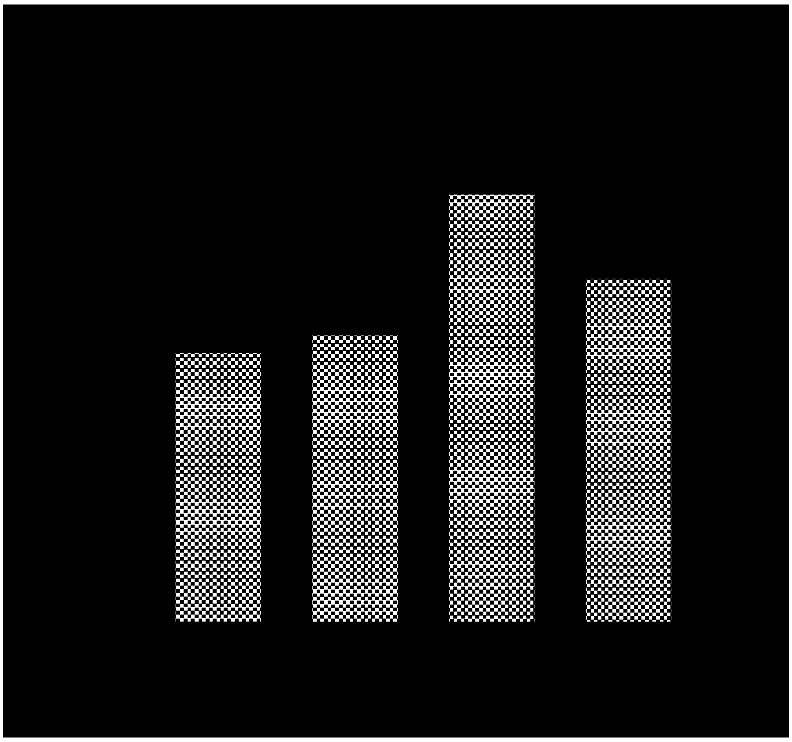


Figure 3.The conditional factor of the fish treated with PTX, Fermacto (as positive control) or placebo (the control) following 45 days of the experiment. Data are represented as means and SEM. Asterisks indicate statistical significance compared to the control (:*p*<0. 01; ***:*p*<0. 001).**

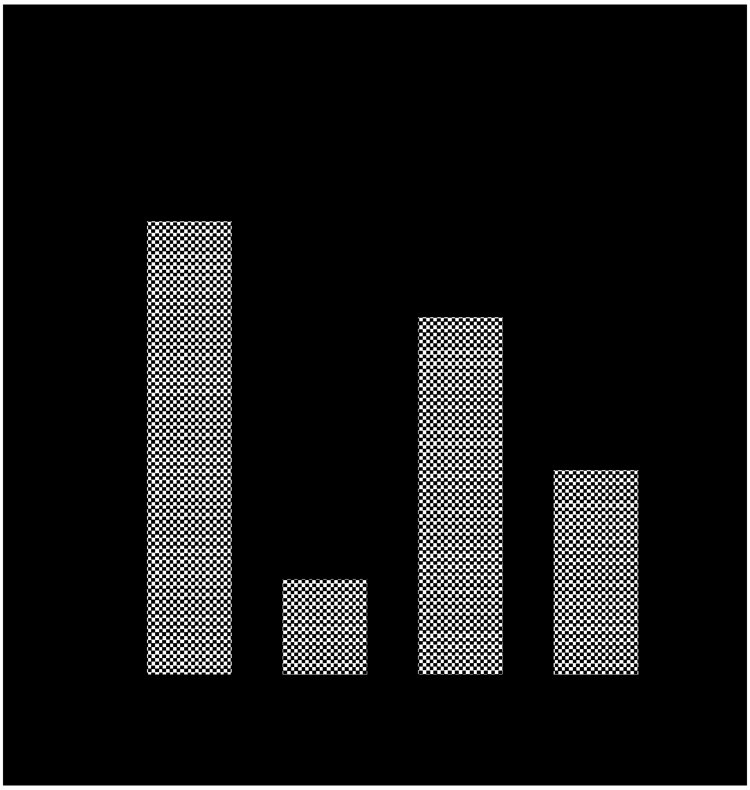


Figure 4: The effect of PTX or Fermacto (used as positive control) on the meat fat of carps following 45 days of the experiment. Data are represented as means and SEM. Statistical significance compared to the control is indicated using asterisks (*p*<0. 001).

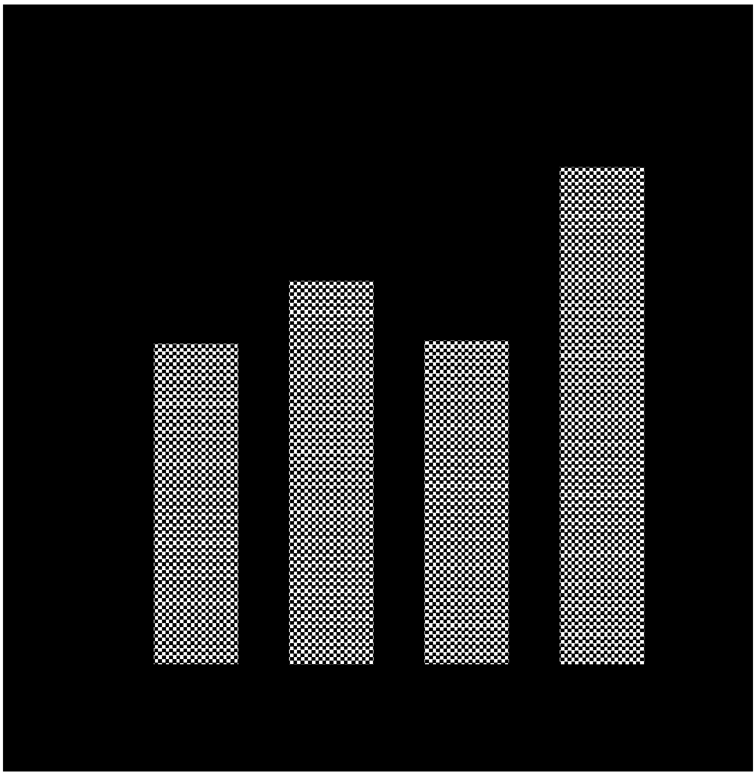


Figure 5.The effect of 45 days of treatment with PTX, Fermacto (used for the positive control group) or placebo on the meat protein of carps. Data are represented as means and SEM. The asterisks indicate statistical significance compared to the control (:*p*<0. 01).**

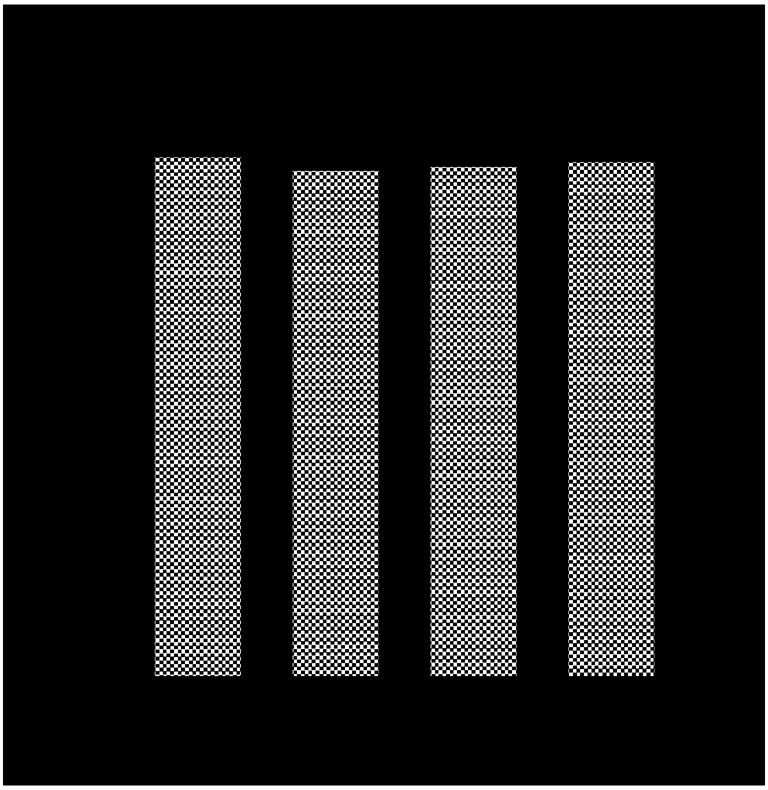


Figure 6.The percentage of meat dry matter following 45 day of treatment with PTX or Fermacto (used as positive control) compared to the control. Data are shown as means and SEM.

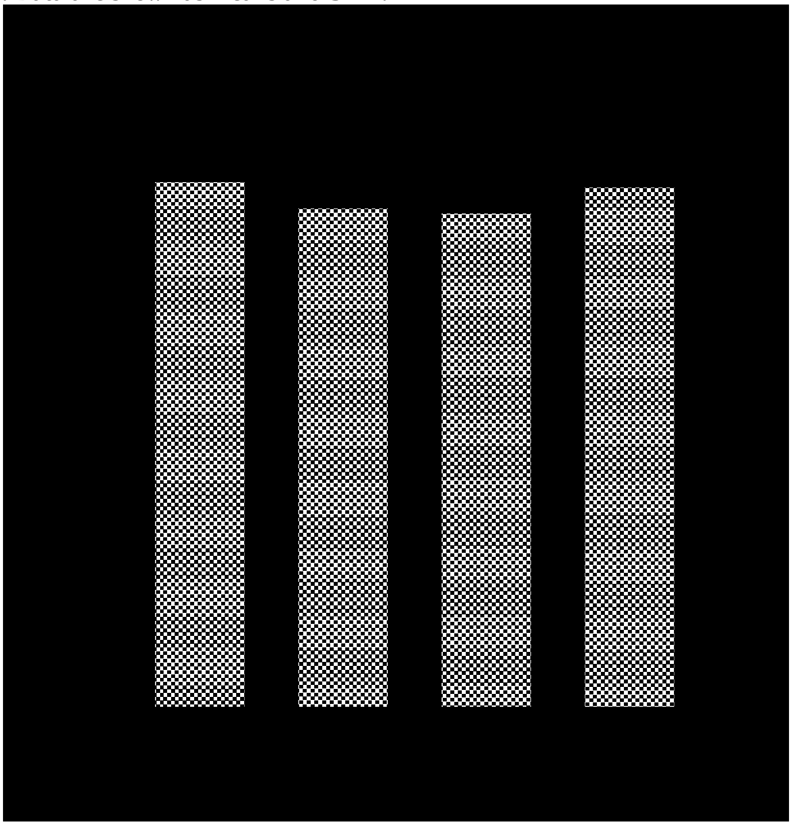


Figure 7.The effect of PTX or Fermacto (used as positive control) on meat ash compared the control following 45 days of the experiment. Data are represented as means and SEM.

The lowest percentage of meat protein was observed in the control group (Figure 5). It increased significantly by 54% with the highest dose of the supplement.

The average meat dry matter and ash in the test groups were not statistically different compared to the control group (Figures 6 and 7).

Discussion

Although different bacteria and even fungi have been used as probiotics for aquaculture, most studies have focused on *Bacillus* species. A combination of five different *Bacillus* species has been successfully used in different aquatic species and is commercially supplied to the market as Protexin Aquatech. This probiotic has shown beneficial effects on the growth of rainbow trout larvae (Jafaryan *et al.*, 2008), Indian white shrimp, *Fenneropenaeus indicus* (Ziaei-Nejad *et al.*, 2006) and *Acipenser persicus* larvae (Jafarian *et al.*, 2007). Incorporation of *Bacillus spp.* isolated from common carp ponds into the carp diet significantly increased the growth performance and digestive enzyme activities (Wang and Xu, 2006).

In addition to *Bacillus species*, other microorganisms have also been used as probiotics for common carps. For instance, feeding a yeast probiotic, *Saccharomyces cerevisiae* strain 1026, to young carps has enhanced production performances (Bud *et al.*, 2003). *Streptococcus faecium* M74 has also been used as probiotic and has resulted in higher weights and better feed conversion rates in carps (Bogut *et al.*, 1998).

In the present study, the effect of a multi-strain probiotic on the growth of fingerling carps was studied. The supplement was composed of different bacterial species, four of which were lactobacilli, and also two different yeast species. In this research in which we used two different doses (75 and 150 mg/kg feed) and over a trial duration of 45 days, the supplement resulted in substantial increases (>70%) in the body weights of the treated carps, compared to the control group. Consistently, the treatment had a significant positive effect on the body lengths of the animals. The feed conversion rate was decreased by 62% and the condition factor was improved by 58% in PTX treated animals. Interestingly, the percentage of meat fat declined by up to 54%, while the protein content of the meat almost doubled due to the supplement. The best growth promoting results were observed with 75 mg PTX/kg feed, whereas the most profound effects on meat fat and protein were obtained with 150 mg PTX/kg feed.

In the current research, the prebiotic Fermacto, was administered to the positive control group. This commercial supplement is primarily used in poultry production industry. However, in a recent research (Mazurkiewicz *et al.*, 2008), the effects of three different doses of the supplement (1, 2 and 3 g/kg feed) have been studied on the growth rates of common carps during 50 days experimental period. The best effects were observed in fish receiving the highest dose of the product (3 g/kg feed, that is the same dose used in the current research), where the specific growth rate (2.44%) and FCR (1.21) were significantly higher than those of the control group.

To the best of our knowledge, there is no report regarding the effect of a similar multi-strain probiotic on common carp. However, the same combination of micro-organisms has been used for other aquatic species. For instance, the supplement (especially at 150 mg/kg feed) has shown positive growth promoting effects on rainbow trout larvae (Mohammadi Azarm *et al.*, 2004). This happened although different doses of the same supplement, in another study, failed to show any significant differences on the growth rates of rainbow trout (average body weight: 470mg) fed a diet based on frozen *Daphnia magna* (Ahmadvand *et al.*, 2012). The effects of this probiotic on the growth and

performance of the ornamental fish, Oscar (*Astronotus ocellatus*), fingerlings have also been investigated (Firouzbakhsh *et al.*, 2011). In the latter experiment, the fish receiving the supplement at 150 mg/kg feed, showed significantly higher final weights and body weight gains at the end of 60 days of experiment. In another study, the probiotic has failed to induce significant changes on the growth parameters of Siberian sturgeon (*Acipenser baerii*) after 8 weeks of treatment (Mahmoudi *et al.*, 2012).

As far as the authors are concerned, there is no report regarding the effect of this multi-strain probiotic on meat quality of common carp. However, the same probiotic has been tested on carcass quality of Siberian sturgeon following 8 weeks of trial period (Mahmoudi *et al.*, 2012). Accordingly, significantly higher carcass protein levels were observed in fish receiving the supplement at 0.5-1 g/kg diet. The carcass crude lipid levels were also significantly lower in these groups compared to the control. Although much higher doses of the probiotic had been fed to the animals, and despite the differences in the species, the results are consistent with the present study. In contrast to the present results, addition of *Bacillus subtilis* to common carp feed did not cause significant changes in the meat protein or the meat fat (Král *et al.*, 2013). This suggests a better meat quality with the present probiotic (at 150mg/kg), due to improved protein efficiency.

In conclusion, the present results clearly demonstrate the growth promoting effects of the multi-strain probiotic on common carps. The probiotic seems to improve the body weight, body length, condition factor and FCR in treated carps. In addition, the supplement may increase meat protein and decrease meat fat in this aquatic animal.

Acknowledgements

This study was sponsored by the School of Veterinary Medicine, Ferdowsi University of Mashhad (project No: 358). The supplement was a gift from Nicotech Company. We are also grateful to Mr. Jafari for his technical assistance.

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اثرات فزاینده رشد یک پروبیوتیک چند سویه ای بر ماهی کپور انگشت قد

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دریافت مقاله: 1394/9/15 پذیرش نهایی: 1395/2/26

چکیده

پروبیوتیک‌ها اثرات سودمندی بر روی پارامترهای رشد حیوانات مولد غذا نشان داده‌اند. در این پژوهش اثرات محرک رشد یک پروبیوتیک چند سویه‌ای در ماهی کپور مورد بررسی قرار گرفت. بر این اساس 200 قطعه ماهی با وزن متوسط 7-6 گرم به طور تصادفی در 4 گروه آزمایشی قرار گرفتند. گروه اول به عنوان شاهد در نظر گرفته شد و دارو نما دریافت نمود. گروه دوم به عنوان کنترل مثبت در نظر گرفته شد و به میزان 3 گرم در هر کیلوگرم غذا فرمکتو دریافت نمود. دو گروه دیگر به ترتیب به میزان 75 و 150 میلی گرم به ازای هر کیلو گرم غذا یک پروبیوتیک چند سویه‌ای (PTX) دریافت کردند. در پایان این پژوهش 45 روزه، تمامی ماهیان وزن گیری شده و طول بدن آن ها اندازه گیری شد. همچنین میزان پروتئین، چربی، خاکستر و ماده خشک گوشت ماهیان در تمامی گروه های آزمایشی ($n = 6$ در هر گروه آزمایشی) اندازه گیری شد. میانگین وزن و طول ماهیان و شاخص وضعیت بدن در گروهی که پروبیوتیک را به میزان 75 میلی گرم در هر کیلوگرم غذا دریافت کرده بود به طور معنی داری نسبت به دیگر گروه‌ها بیشتر بود. در همین راستا، ضریب تبدیل غذایی نیز در گروه‌هایی که PTX دریافت کرده بودند، کمتر بود. درصد چربی گوشت در گروه‌هایی که پروبیوتیک به میزان 150 میلی گرم به ازای هر کیلوگرم جیره و یا فرمکتو دریافت کرده بودند به طور معنی داری نسبت به گروه شاهد کمتر بود. میزان پروتئین گوشت ماهیانی که به میزان 150 میلی گرم به ازای هر کیلوگرم جیره PTX دریافت کرده بودند به طور معنی داری بیشتر بود. مقادیر خاکستر و ماده خشک گوشت در بین گروههای مختلف آزمایشی هیچ گونه تفاوت آماری نشان نداد. این پژوهش پیشنهاد می کند، PTX بر روی بهبود ضریب تبدیل غذایی و اضافه وزن ماهی کپور معمولی موثر است.

واژگان کلیدی: پروبیوتیک چند سویه‌ای، فرمکتو، ماهی کپور معمولی، ضریب تبدیل غذایی